

Incidence Study of Spores of *Clostridium botulinum* in Convenience Foods

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The objective of this study was to gather data on the incidence of *Clostridium botulinum* spores in selected consumer-convenience food products. The incidence of spores of *C. botulinum* in 100 samples of each of four categories of commercially available convenience foods was determined. These categories included (i) "boil-in-the-bag" foods, (ii) vacuum-packed foods, (iii) pressurized foods, and (iv) dehydrated and freeze-dried foods. Of the 400 samples analyzed, one was found to contain the spores of *C. botulinum*. This occurred in vacuum-packed frankfurters and was identified as type B.

The past decade has seen an increase in the availability and use of vacuum-packed convenience foods. Data on the incidence of *Clostridium botulinum* in natural, noninoculated, vacuum-packed foods are limited. Greenberg et al. (6) surveyed the incidence of mesophilic clostridium spores in raw pork, beef, and chicken in processing plants in the United States and Canada. *C. botulinum* type C was isolated from a chicken sample at a level of 5.33 clostridia per g. Vacuum-packed products such as meat, fish, oysters, cheese, vegetables, and oriental-type foods were examined for *C. botulinum* by Taclindo et al. (16). *C. botulinum* type B toxin was found in one of 73 luncheon meats, and type C organisms and toxin were found in one of four unshucked oysters. The frequency of *C. botulinum* contamination of freshly caught and eviscerated chubs was found by Pace et al. (11) to be approximately 13 to 14%. Of 858 samples of freshly smoked chubs processed for 30 min to an internal temperature of 82.2 C (180 F), 10 were found to be contaminated with *C. botulinum*. The 10 isolates were composed of one type B and nine type E. Insalata et al. (7) analyzed 100 vacuum-packed fish that had been stored at 10 C for 28 days for botulinum toxin. After enrichment, 10 samples were found to contain type E exotoxin.

Most of the work reported has been abuse studies with inoculated packs. Perry et al. (12) tested for the presence of spores of *C. botulinum*

and for toxicity in frozen-pack vegetables that were left standing in unopened containers for 2 days at room temperature. Although the vegetables were spoiled, none produced toxin. Working with inoculated turkey rolls, Midura, et al. (10) found that an inoculum equivalent to one spore per gram of food was the minimal number needed for toxin production under anaerobic incubation at 30 C. Liver sausage inoculated with 5,000 spores/g of *Clostridium parbotulinum* type A and incubated at 30 C allowed formations of toxin in 9 to 23 days. The sausage contained 2.5% salt and 0.1% sodium nitrate; increasing these concentrations and lowering the incubation temperature increased the time required for toxin formation (15). The rate of toxin formation and putrefaction in inoculated comminuted pork and beef was studied by Greenberg and Silliker (5). They found that putrid smells and toxin occurred within 24 hr at 37 C (99 F) in such vacuum-packed meats. Cann et al. (1) inoculated vacuum-packed fresh herring with 100 spores per pack. After storage for 15 days at 5 C, toxin was formed. Fish inoculated with 10⁶ *C. botulinum* type E spores, smoked for 30 min to an internal temperature of 82.2 C (180 F), and sealed in plastic bags produced toxin after 7 days at 20 to 25 C (2).

The objective of this study was to gather data on the incidence of *C. botulinum* spores in selected convenience food products.

MATERIALS AND METHODS

Samples. Four types of commercially available convenience foods were used: (i) "boil-in-the-bag" foods, (ii) vacuum-packed foods, (iii) pressurized foods, and (iv) dehydrated and freeze-dried foods.

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In each category, 100 samples were analyzed. A category was comprised of 10 different food prototypes (Table 1) from various manufacturers. Ten samples per food prototype were analyzed, a total of 400 samples.

Preparation of samples. All samples were divided into two equal portions. One portion was used for the unheated phase and the other for the heated phase. All portions equaled 175 ml after blending with Duff's medium (4). Pouches of boil-in-the-bag vegetables were cut in half while frozen. Each half was blended in 50 ml of Duff's medium. Dehydrated foods were reconstituted with lukewarm water and drained according to the manufacturer's specification. The vacuum-packed, pressurized, and reconstituted foods were divided into two equal portions, and each half was blended separately in 100 ml of Duff's medium.

Treatment of samples. One portion of a sample was added to an equal volume (175 ml) of Duff's medium in a sterile pint jar. The second portion was added to 175 ml of Duff's medium in a sterile pint jar and heated at 60 C for 15 min in a rotary water bath. It took approximately 3 to 6 min for the temperature to reach between 59 to 63 C. Additional quantities (2 to 5 ml) of Duff's medium were added to the jars, when necessary, to assure a minimal headspace. Samples were incubated at 28 C for 7 days in anaerobic jars. After 7 days of enrichment, the samples were frozen at 0 C until analyzed for toxins.

Initial toxin analysis. Samples were centrifuged at 2,500 rev/min at 4 C for 30 min. The supernatant fluid was decanted into sterile jars. A sample (10 ml) of the supernatant fluid was trypsinized at 37 C (3) for 1 hr. One-half of the trypsinized sample was diluted 1:10 with gelatin phosphate buffer. The other half was left undiluted. A portion (3 ml) of the supernatant fluid (untrypsinized) was diluted 1:10 with gelatin

phosphate buffer. Another 3 ml-portion was used undiluted.

Two mice were inoculated with 0.4 ml from each treatment. The mice were observed for 96 hr for botulogenic symptoms and deaths. The remaining supernatant fluid was frozen for further toxin confirmation.

Antitoxin confirmation. Samples that killed mice were taken from frozen storage. These were retested with antitoxin to confirm the presence of *C. botulinum* exotoxin and to identify the type. The highest dilution of the sample that produced death during the initial toxin analysis was used for testing with antitoxin.

Preparation. The supernatant fluid was divided into three equal portions. To the first portion, 0.1% trypsin was added and incubated at 37 C for 1 hr. The second portion received no trypsinization. The third portion was boiled for 10 min.

TABLE 2. Confirmation in vacuum-packed frankfurters positive for *C. botulinum* spores, type B^a

Treatment	Samples		
	No dilution	Diluted 1:5	Diluted 1:10
Boiled	00		
Untrypsinized	++	++	++
Trypsinized	++	++	++

^a Results are expressed in terms of deaths (+) and survivals (0) of the test mice. Mice tested with toxins A, B, and E resulted in two deaths, two survivals, and two deaths, respectively.

TABLE 1. Food prototypes examined for spores of *C. botulinum*

Boil-in-the-bag		Vacuum-packed foods	Pressurized foods	Dehydrated foods
Vegetables	Fish and poultry			
Asparagus cuts and tips, hollandaise style	Lobster newburg	Ham slices	Cake decorator, brands A, B, and C	Freeze-dried shrimp
Baby brussels sprouts	Shrimp newburg	Bologna	Synthetic whipped cream, brands A, B, C, D, and E	Freeze-dried king crab
Cauliflower in cheese sauce	Crab newburg	Head cheese	Salad dressing	Shrimp salad mix
Cut leaf spinach in butter sauce	Chicken pot pie filling	Chicken slices	Cheese spread	Crab salad mix
Broccoli spears in butter sauce	Chicken a la king	Turkey slices		Tuna salad mix
		Frankfurters		Chicken salad mix
		Edam cheese		Instant chicken gravy mix
		Cheddar cheese		Beef dices
		Pork slices		Mushrooms, A and B
		Liverwurst slices		

Inoculation. Samples of 1.2 ml of the trypsinized, untrypsinized, and boiled samples were mixed with 0.3 ml of monovalent antitoxin types A, B, and E, respectively (standard antitoxin obtained from the Public Health Service; 0.1 ml equal to 1 IU). These were allowed to react at room temperature for 30 min. Two mice were injected with 0.5 ml of each type of antitoxin and treatment sample. The toxin type was indicated by the antitoxin which protected the mice from death for 4 days from the time of inoculation.

RESULTS

One out of the 400 samples analyzed was positive for *C. botulinum* spores. Type B toxin was identified in vacuum-packed frankfurters. The unheated samples and both the trypsinized and untrypsinized portions were positive for botulinum exotoxin (Table 2).

DISCUSSION

Detection of *C. botulinum* spores in one of 400 samples examined demonstrated a low incidence rate. However, the isolation of type B spores from vacuum-packed frankfurters emphasized the need to refrigerate and to prevent abuse of vacuum-packed meats to eliminate a possible health hazard to the consumer. Reports on the risk of the development of toxin in vacuum-packed foods vary. According to Johannsen (8), the risk of toxin formation is dependent primarily on the product itself and not the packing. Kautter (9), in his study of smoked ciscoes inoculated with type E spores and held at 30 C, found that packages open to the air became toxic as rapidly as those in plastic, airtight packages. On the other hand, Thatcher et al. (17) reported that anaerobic packaging of foods may increase the food poisoning hazard by allowing the production of botulinum toxin, which would occur at refrigerator temperatures (13, 14).

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